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1-Desamino-8-Arginine-Vasopressin Corrects the Hemostatic Defects in Type 2B von Willebrand's Disease

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DDAVP is effective treatment in most types of von Willebrand's disease; however, in type 2B von Willebrand's disease the use of DDAVP has been contraindicated due to DDAVP-induced thrombocytopenia. Several reports have confirmed the thrombocytopenic effects of DDAVP and the presence of circulating platelet aggregates in type 2B von Willebrand's disease. We have infused three type 2B patients with DDAVP. The three patients had different mutations of their vWf. All three patients had a missense mutation which resulted in a single amino acid substitution in the disulfide loop of the A1 domain. Administration of 20 μg of DDAVP resulted in significant elevations of factor VIII, vWf antigen, and ristocetin cofactor levels. In contrast to other studies, DDAVP did not induce or enhance thrombocytopenia in these three patients. When blood was obtained by fingerstick and diluted into sodium oxalate (Unopette®) or EDTA (Microvette®), the platelet counts did not change over 4 hr. In contrast, blood collected directly into evacuated tubes containing sodium citrate, lithium heparin, or EDTA consistently demonstrated varying degrees of thrombocytopenia and platelet clumping. We also observed a shortening of the pre-infusion bleeding time over the 4 hr period. All three patients have been studied twice and each has shown consistent results. DDAVP appears to be a useful form of treatment in type 2B vWd. © 1996 Wiley-Liss, Inc.*

Key words: DDAVP, type 2B von Willebrand's disease, thrombocytopenia

INTRODUCTION

1-desamino-8-D-arginine-vasopressin (DDAVP) infusion increases the plasma levels of factor VIII, von Willebrand factor antigen, and ristocetin-cofactor activity in normal individuals, patients with hemophilia A, and most patients with type 1 von Willebrand's disease (vWd). In patients with type 1 vWd with normal platelet vWf, the prolonged bleeding time is usually corrected [1–6]. Some studies have described hemostatic improvement after DDAVP in type 2A vWd, while others studies have shown a lack of benefit in type 2A vWd [7, 8]. DDAVP therapy is not considered a useful and/or safe form of therapy in type 2B vWd since its administration has been associated with thrombocytopenia and circulating platelet aggregates [9–13]. To determine the effect(s) of DDAVP on platelet counts in type 2B vWd, we have administered DDAVP to three type 2B vWd patients with different missense mutations and examined their hemostatic response(s).

MATERIALS AND METHODS

The three patients described in this study have multiple affected family members who have similar coagulation abnormalities. At the time of these studies the ages of the three patients were 32, 41, and 46 years old. Patient 1 is a male and patients 2 and 3 are females. In the family of patient 1, the disease has been diagnosed in three generations, in patient 2, for two generations and in patient 3, for three generations. The molecular defect in the vWf gene has been identified in these three patients. Each patient has a single missense mutation resulting in a single amino acid substitution in the disulfide loop of the A1 domain encompassing amino acids Cys509–Cys695. The

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substitution in these three patients are: patient 1, Arg578-Gln; patient 2, Arg545-Cys; and Patient 3, Val553-Met [14].

The patients were aware of the experimental nature of the study and the possibility of adverse effects. The patients gave their informed consent. All experiments were performed according to the Declaration of Helsinki. DDAVP (Stimate, Revlon-Armour, Berkeley Heights, NJ) was administered at a dose of 0.3 μg/kg body weight. Regardless of the patient's weight, the maximum dose administered was 20 μg. DDAVP was added to 50 mL of normal saline and infused intravenously over a 30 min period. Before, during, and after the infusion, blood pressure and pulse were checked every 30 min in the arm that was not used for venipuncture or bleeding time determinations.

Blood was collected by venipuncture into evacuated tubes containing either lithium heparin, sodium citrate, or EDTA as the anticoagulant. Whole blood and fingerstick samples were obtained before and 30, 60, 120, and 240 min after the DDAVP infusion ended. The fingerstick platelet counts were obtained with the Unopette® (Becton Dickinson, Rutherford, NJ) or the Microvette® (Sarstedt, Newton, North Carolina). Blood samples were collected by venipuncture with a 19-gauge needle in a polypropylene syringe using a two-syringe technique. The blood in the first syringe was used for platelet counts employing different anticoagulants and methods of platelet enumeration. The blood collected in the second syringe was used for coagulation studies. The polypropylene tubes used for the coagulation studies contained sodium citrate 3.2% (final concentration 10.9 mM). The blood was centrifuged at 3,000g at 4°C for 15 min and the plasma was separated. Factor VIII activity was assayed on fresh samples by a one-stage method based on the partial thromboplastin time as previously described [3]. The plasma was frozen at −70°C and tested within 7 days for vWf antigen and ristocetin cofactor activity (RCoF).

vWf antigen (Ag) was assayed by electroimmunodiffusion, the vWf (RCoF) activity was measured using formalinized fixed platelets as previously described, and the multimeric structure of vWf was analyzed by agarose gel electrophoresis in the presence of sodium dodecyl sulfate as previously described [3]. The vWf multimers were identified after incubation in the agarose gel with ^{125}I affinity-purified rabbit anti-human vWf antibody and subsequently developed by autoradiography. Bleeding times were performed with a modified Ivy technique using a standardized template technique (Simplate® Organon Technika, Durham, NC) prior to the infusion of DDAVP and 1 and 4 hours after the infusion had ended.

Aggregation studies were performed using a Chronolog lumiaggregometer (Chronolog, Havertown, PA). Platelet-rich plasma was obtained from citrated whole blood (final concentration 10.9 mM) by centrifugation at 750g for 3 min at room temperature. Platelet poor plasma, obtained by centrifugation at 2,000g for 10 min, was used to adjust the platelet count to 200,000/μl. For inhibition studies 50 μl of monoclonal antibody was incubated with 400 μl of platelet-rich plasma for 10 min at 37°C (final concentration 1.0–2.5 μg/ml).

Two murine monoclonal antibodies were utilized in platelet aggregation studies. The antibody F302D9 is an IgGγ produced in our laboratory that inhibits ristocetin-induced vWf binding to GPIb. The other monoclonal antibody 6D1 (a gift of Barry Coller) inhibits the vWf binding site on GPIbα.

RESULTS

The three patients offered no complaints before, during, or after the DDAVP infusion and their vital signs remained stable throughout the 5 hr period of observation. The three type 2B patients had similar coagulation results (Table I) except for their bleeding times and platelet counts prior to the DDAVP infusion. The factor VIII level was reduced in the three patients, and in two patients the RCoF was decreased. Bleeding times were prolonged in all three patients (12.5 min to >30 min, normal <8.5 min). All three patients demonstrated enhanced ristocetin-induced platelet aggregation at low ristocetin concentrations (0.2–0.4 mg/ml). Each patient had varying degrees of spontaneous platelet aggregation (SPA) of their platelet-rich plasma which was inhibited by either an anti-vWf monoclonal antibody (302D9), which inhibited ristocetin-induced vWf binding to GPIb, or an anti-GPIb monoclonal antibody (6D1) which inhibited ristocetin-induced von Willebrand binding to GPIbα. Each of the two monoclonal antibodies totally inhibited the SPA in the three patients at concentrations varying from 1.0–2.5 μg/ml.

The multimeric analyses of the plasma vWf in each patient showed an absence of the high molecular weight multimers and reduced intermediate sized multimers prior to DDAVP administration. Within 30 min after the completion of the DDAVP infusion, each patient's plasma contained greater amounts of vWf antigen and higher molecular weight multimers than prior to the DDAVP; however, none of the patients had a normal distribution of vWf multimers. The platelet vWf multimeric structure pre- and post-DDAVP revealed a normal complement of high, moderate, and low molecular weight multimers (data not shown).

After the DDAVP infusion, the ristocetin cofactor and factor VIII activities were increased in all three patients. The peak of factor VIII, ristocetin cofactor activity and vWf antigen occurred between 30 and 120 min after the end of the DDAVP infusion. In patient 1, the factor VIII level rose to 1.68U/ml and the ristocetin cofactor also

TABLE I. DDAVP in 2B vWd

Patients[a]	Pre	30 min	60 min	120 min	240 min
vWd1					
Factor VIII	0.46	1.64	1.68	1.39	0.94
RCof	0.50	1.90	1.68	0.99	0.97
vWf Ag	0.49	1.90	1.83	1.15	1.27
B.T.	12.5	—	9.0	—	8.5
vWd2					
Factor VIII	0.31	1.37	0.81	0.76	0.63
RCof	0.28	0.89	0.86	0.65	0.52
vWf Ag	0.39	1.43	1.43	1.05	0.86
B.T.	33.5	—	5.5	—	5.5
vWd3					
Factor VIII	0.50	1.23	1.37	0.91	0.90
RCof	0.32	0.77	0.78	0.82	0.47
vWf Ag	0.67	1.20	1.40	1.23	1.18
B.T.	>30	—	>30	—	11

[a]Normal ranges: F.VIII, 0.55–1.44 U/ml; RCoF, 0.48–1.44 U/ml; vWf Ag, 0.50–1.50 U/ml; Bleeding time, <8.5 min.

TABLE II. Platelet Counts in Type 2B vWd Treated With DDAVP Patient 1

Anticoagulant	PRE[c]	30 min	60 min	120 min	240 min
EDTA[a]	160	144	145	146	146
NH_4 Oxalate Unopette® (Fingerstick)[b]	175	130	175	172	148
Sodium citrate[a]	79	102	117	126	123
Heparin[a]	48	96	69	98	47
EDTA Microvette® (Fingerstick)[a]	186	116	143	132	128

[a]Analysis by Coulter STKS.
[b]Analysis by phase microscopy.
[c]Platelet count × $10^3/\mu l$.

rose to 1.68U/ml. In patient 2, the factor VIII peaked at 1.37U/ml and the ristocetin cofactor at 0.89U/ml within 30 min after the DDAVP infusion. In patient 3, the factor VIII and ristocetin cofactor rose to 1.37U/ml and 0.78U/ml, respectively, 60 min post-DDAVP (Table I).

The bleeding time, which had been prolonged (12.5 min) before DDAVP infusion, was shortened to 9.0 min in patient 1, 1 hr after the end of the infusion and to 8.5 min at 4 hr. In patient 2, the bleeding time was shortened from 33.5 min to 5.5 min at 1 and 4 hours after DDAVP infusion. In patient 3, the bleeding time remained >30 min at 1 hr, but at 4 hr it had shortened to 11 min.

The pre-DDAVP platelet count performed by a variety of techniques showed variable results in the three patients (Tables II–IV). The mean platelet count(s) employing lithium heparin, sodium citrate, or EDTA as the anticoagulant in glass or plastic tubes showed marked variability with the formation of platelet clumps and decreased numbers of platelets. In contrast, in all three patients, one or both fingerstick platelet counts showed higher values after DDAVP than with any of the other methods employed (Tables II–IV).

DISCUSSION

DDAVP has been a controversial form of therapy in type 2B vWd. This, in part, has emanated from the reports of Holmberg et al. and other investigators that described thrombocytopenia and platelet aggregates in samples of their 2B patient's blood after DDAVP infusion [9–11], and by the rare association of thrombotic events occurring after DDAVP administration to normal individuals or to patients undergoing cardiovascular surgery [15–18]. Each of our 2B vWd patients received one or two DDAVP infusion(s) without any ill effects or morbidity. In each of the three patients post-DDAVP, the factor VIII and ristocetin cofactor levels rose to normal. In two patients the prolonged bleeding times were shortened at 1 and/or 4 hr after the DDAVP infusion and in the third patient it shortened the bleeding time from >30 min to 11 min at 4 hr post-DDAVP. Comparison of the platelet counts prior to, during, and after DDAVP infusion showed considerable variation. This was in part due to the anticoagulant employed, and the type of sample collection, e.g., whole blood or fingerstick. The platelet counts obtained with

TABLE III. Platelet Counts in Type 2B vWd Treated With DDAVP Patient 2

Anticoagulant	PRE[a]	30 min	60 min	120 min	240 min
EDTA[b]	117	72	70	83	100
Heparin[b]	31	TC	45	35	68
Sodium citrate[b]	77	TC	50	54	78
NH_4 Oxalate Unopette® (Fingerstick)[c]	121	119	77	108	115
EDTA Microvette®[b]	117	72	70	83	100
EDTA Microvette® (Fingerstick)[c]	134	117	77	108	135

[a]Platelet count × $10^3/\mu l$; T.C., platelets too clumped to count.
[b]Analysis by Coulter STKS.
[c]Analysis by phase microscopy.

TABLE IV. Platelet Counts in Type 2B vWd Treated With DDAVP Patient 3

Anticoagulant	PRE[a]	30 min	60 min	120 min	240 min
EDTA[b]	36	5	56	20	37
Heparin[b]	37	11	12	26	28
Sodium citrate[b]	33	3	11	23	32
NH_4 Oxalate Unopette® (Fingerstick)[c]	52	31	29	36	39
EDTA Microvette® (Fingerstick)[b]	56	27	32	37	44

[a]Platelet count × $10^3/\mu l$.
[b]Analysis by Coulter STKS.
[c]Analysis by phase microscopy.

the Unopette® or Microvette® were consistently higher than the counts obtained using other collection systems (Tables II–IV).

Comparative studies of our three 2B patients to other reported patients with the same molecular defect(s) show similar clinical and laboratory findings [14,19–24]. Likewise, our studies of these three type 2B vWf mutants agree, in general, with the published results of other investigators using recombinant vWf mutants associated with type 2B vWd [25–27].

Other studies of the administration of DDAVP in type 2B vWd have provided varied results. Mannucci reported poor bleeding time responses and thrombocytopenia [2,4]. Casonato et al. [12] have reported that DDAVP infusions in type 2B vWd patients resulted in platelet aggregates and thrombocytopenia. In contrast, Fowler et al. used DDAVP for 7 days to treat a 2B patient before and during surgery [28]. They reported the presence of platelet aggregates and the development of thrombocytopenia, but they did not find any evidence of organ compromise, hemorrhage, or thrombosis. The authors concluded that DDAVP could be used to treat type 2B patients. Casonato et al. reported "pseudo thrombocytopenia" in their type 2B patients treated with DDAVP. To date, no one has described any deleterious clinical effects in the type 2B vWd patients treated with DDAVP. Nevertheless, the aforementioned laboratory and clinical observations have lead to the dictum that DDAVP is contraindicated in Type 2B vWd [9–11].

Our studies indicate that fingerstick platelet counts collected in EDTA with Microvettes®, or in sodium oxalate with Unopettes®, were higher than the other methods tested. Whole blood collected in sodium citrate, lithium heparin, or EDTA resulted in decreased platelet counts and "clotted" or clumped platelet samples (Tables II–IV). Studies of platelet counts after DDAVP infusion with the fingerstick devices Microvette® and Unopette® gave results which were similar to the preinfusion counts and were higher than the other systems tested. This would suggest that when the blood is rapidly diluted in-vitro, platelet aggregate formation is minimal or non-existent. In contrast, when the blood sample is not diluted, e.g., whole blood studies, platelet aggregates are observed. Since the platelet aggregates would not be counted as platelets by most electronic particle counters or enumerated by phase microscopy, this would further add to the "thrombocytopenia" attributed to DDAVP in type 2B vWd.

The clinical importance of this study is the finding that the administration of DDAVP in three type 2B vWd patients partially or totally corrected the prolonged bleeding time, although the platelet counts were variable and did not necessarily correlate with the bleeding time. This raises the possibility that DDAVP may affect the vessel wall and/or platelets in a manner which enhances platelet adhesion to the subendothelial surface [29]. The work of Kroll et al. [30], Cattaneo et al. [31], and Murata et al. [32] support this hypothesis. Kroll et al. [30] have shown that vWf binding to GPIb initiates the intracellular pathway of platelet activation. This, in turn, results in the

rise of cytoplasmic $[Ca^{2+}]_i$, synthesis of thromboxane A_2, phosphatidic acid, and activation of protein kinase C. Cattaneo et al. have described DDAVP potentiation of shear-induced platelet aggregation [31], while Murata et al. have observed that low shear stress can initiate vWf-dependent platelet aggregation [32].

Studies by Ikeda et al. [33] stressed the importance of vWf binding to GPIb in high shear stress-induced platelet aggregation. Murata et al. showed that the blood of type 2B vWf patients at low shear stress (18 dyne/cm^2) showed enhanced vWf-dependent platelet aggregation as compared to normals [32]. This may in part explain i) the observation(s) of the presence of platelet aggregates in the blood of type 2B vWd and ii) the relative lack of thrombotic or microvascular complications in type 2B vWd. The platelet aggregates that form and circulate may not be able to withstand the high shear forces that occur in the microcirculation. The "thrombocytopenia" associated with DDAVP administration appears to be primarily an in-vitro phenomena. In our experience, DDAVP improved clinical hemostasis and shortened the bleeding time in type 2B vWd.

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